



Yield and chemical constituents of Dragon's head (*Lallemantia iberica* Fisch. et Mey) as influenced by planting date and spacing

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Abstract

Objective: Agricultural strategic decisions, like planting dates and spacings, have direct impacts on sustainable crop production. This experiment aimed to investigate the effects of different planting dates and spacing on grain yield and chemical constituents of dragon's head (*Lallemantia iberica* Fisch. et Mey).

Methods: A split-plot design based on a randomized complete block design with three replications was carried out to study the influence of planting dates (March 18, April 2, and April 18) and spacing (20×3, 20×5, and 30×2 cm inter- and intra-row spacing) on *L. iberica* at Urmia University, Iran, in 2018. Seeds of dragon's head were provided by the Agricultural Research, Education, and Extension Organization, Urmia, Iran. Seeds were sown on three specified planting dates by hand at a depth of 2 cm in plots of 150×200 cm. Then, they were covered with a thin layer of soil.

Results: The maximum grain yield (520 kg/ha), grain oil percentage (31.84%), and grain oil yield (165 kg/ha) were obtained from the March 18 planting date with 30×2 cm spacing. Also, the highest biomass (3796 kg/ha), essential oil percentage and yield (0.34% and 13.17 kg/ha, respectively), mucilage percentage and yield (15.30% and 70 kg/ha, respectively) were observed on the March 18 planting date. The maximum percentage of grain nutrients (NPK) was obtained from the earliest planting date and the planting spacing of 20×5 cm. The highest percentage of total unsaturated fatty acids against the lowest saturated fatty acids (stearic and palmitic acid) belonged to March 18. However, the highest percentage of some of the important compounds in the *L. iberica* essential oil, including thymol, caryophyllene oxide, and phytol, was obtained on April 18.

Conclusion: Early planting on March 18 was a better date for *L. iberica*, which gave a higher grain yield than other planting dates. Mutually, late sowing reduced the nutrient uptake, biomass, and some quality traits. Also, spacing of 30×2 cm with lower plant density showed better results in terms of plant quality.

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Introduction

Dragon's head (*Lallemantia iberica* Fish. et Mey.) is an annual herb of flowering plants in the Lamiaceae family, originating in the Caucasian region. *L. iberica* is a valuable species because of biologically active compounds such as polysaccharides, soluble fibers, mucilage, proteins, fixed oils, and essential oils; also, all plant parts can be economically used. It is mainly cultivated for seeds that contain about 30-38% edible oil (*Lallemantia* oil) with a very high content of linolenic acid. Seed mucilage may act mainly as energy reserves and is used in the treatment of the nervous system, hepatitis, and also as a general tonic, aphrodisiac, and expectorant. The major constituents of the dried aerial parts essential oil are p-cymene (22.1%), isophytol (19.8%), T-cadinol (11.1%), 3-octanol (8.1%), caryophyllene oxide (7.4%), and terpinen-4-ol (5.7%) (Heydari and Pirzad 2020a, b; Heydari and Pirzad 2021).

Selecting the correct planting date and spacing plays an important role in the performance and consequently, the yield of medicinal and aromatic plants (Ekren 2021). Planting before or later than the optimal sowing date can result in the reduction of yield and quality because photoperiodism controls the number of days to flowering and available time for vegetative growth and development (Amraei 2022). The influence of planting dates on medicinal plants, such as *L. iberica*, has been indicated by Badawy *et al.* (2013).

Plant spacing also plays a key role in influencing the microclimate for better plant growth and response to environmental factors and management practices (Özyazic 2022). A proper plant spacing improves the accessibility of light intensity and nutrients (Gopichand *et al.* 2013). The impact of plant spacing on *L. iberica* has been reported by Badawy *et al.* (2013). To explore the best production scheme of dragon's head, this experiment was carried out to investigate the effects of different planting dates and spacing and their interaction on yield and chemical constituents of this species.

Materials and Methods

The field experiment was conducted in 2018 at Urmia University, located in the northwest of Iran (37° 39' 24.82" N latitude, 44° 58' 12.42" E longitude, and 1338 m altitude). The results of the chemical and physical analyses of the soil are presented in Table 1.

Table 1. Soil analysis of the experimental site.

Soil depth	Soil texture	Silt (%)	Clay (%)	Sand (%)	T.N.V	Organic matter (%)	N mg/kg	P mg/kg	K mg/kg	EC (dS/m)	pH
0-30	Loam	32	24	44	10.3	0.81	0.09	21	160	0.9	7.9

The experimental design was split-plot based on a randomized complete block design with three replications. Planting dates (March 18, April 2, and April 18) were arranged in main plots, and spacings (20×3 cm, 20×5 cm, and 30×2 cm) in subplots. Before sowing, the land was harrowed, plowed, and rolled. Seeds of dragon's head (with 99% germination and 1000-seed weight of 4 g) were provided by the Agricultural Research, Education and Extension Organization (AREEO), Urmia, Iran. Seeds were sown on three specified planting dates in a loamy soil by hand at a depth of two cm in plots of 150 × 200 cm. Then, they were covered with a thin layer of soil.

At the 75% of the full flowering stage, plants were harvested to measure the biomass (kg/ha) and essential oil constituents (%). When the seeds matured on the plants at the end of the growing season (135, 122, and 110 DAS for March 18, April 2, and April 18 plant dates, respectively), grain yield (kg/ha), grain oil and mucilage (%), oil content (%), and grain's total macro-nutrients content (%) were determined.

The chemical analysis was performed on the oven-dried seed (at 70 °C to a constant weight) and ground into powder. Total macro-nutrients were extracted from the digested samples and were determined as follows: total N by the Kjeldahl analyzer (VAP45, Gerhardt, Germany), p content by the spectrophotometer (Spectronic 20D, Milton Roy, USA), and K by the flame photometer (PEP7, Jenway, Dunmow, UK) (Bryson *et al.* 2014; Seenappa *et al.* 2020; AOAC 2023).

Essential oils were extracted from the dried aerial parts of each plot by hydrodistillation for 3 h, using a Clevenger-type apparatus according to the European Pharmacopoeia. Then, essential oil yield was calculated (Essential oil yield = Essential oil percentage × The yield of dried aerial parts). Then, the water-distilled essential oil was measured by gas chromatography–mass spectrometry (GC/MS) after mixing the three replications. Hewlett-Packard 6890 series was operated in the EI mode at 70 eV, using a DB-5 capillary column (30 m x 0.25 mm, film thickness 0.25 µm), which was programmed as follows: 60 °C for 5 min and then 60°-220 °C at 4°C/min. The carrier gas was He at a flow rate of 2 mL/min. The essential oil components were identified by their retention indices relative to C9-C28 n-alkanes, and by comparing their mass spectra with those of authentic samples or with data from the literature (Sparkman 2005).

For the determination of oil content, the grains (50 g) were air-dried for 72 h at 20 °C and the oil of each sample was extracted with hexane in a Soxhlet apparatus for 8 h. The solvent was removed in a rotary vacuum evaporator to determine the oil content (Zlatanov *et al.* 2012). The oil yield was calculated through the following formula:

$$\text{Oil yield} = \text{Oil percentage} \times \text{Grain yield}$$

Fatty acid profiles were identified by gas chromatography according to the Härtig (2008) protocol. Fatty acid methyl esters were injected by an auto sampler into a Hewlett-Packard 5890A gas chromatograph (Hewlett-Packard, Sunnyvale, CA) with a flame ionization detector. Methyl esters were separated on a 100 m × 0.25 mm i.d. fused silica capillary column (CP-Sil 88; Chrompack, Middleburg, The Netherlands). The carrier gas was hydrogen (ultra-pure), and the let pressure was maintained at 23psi. The injector and detector temperatures were maintained at 250 °C and 255 °C. The initial oven temperature was set at 70 °C, and held for 1 min with a rise of 5 °C per min until 100 °C was reached, and then was held for 2 min. An increase of 10 °C/min was set until 175 °C was reached, and then it was held for 40 min, after which it was elevated to 225 °C with 5 °C/min, and held for 15 min

The Mucilage content of the air-dried grains was determined according to Singer *et al.* (2011). Grains (10 g) were dispersed in the boiling water at 100 °C for 30 min. At the end of the extraction period, the extract was left to cool at room temperature, centrifuged at 4500 g and 5 °C for 30 min. Then, it was filtered through the glass wool, and the filtrate volume was reduced by rotary evaporation. Ethanol was added to precipitate the mucilage from the extract. After 24 h at 5 °C, the precipitate was removed by centrifugation (4500 g for 30 min at 5 °C), then homogenized in a small amount of water, and freeze-dried. Based on the mucilage percentage and grain yield, mucilage yield was calculated through the following formula:

$$\text{Mucilage yield} = \text{Mucilage percentage} \times \text{grain yield}$$

To determine the effect of individual factors and their interaction, Statistical Analysis System (SAS 9.2) was used for the analysis of variance through the general linear model option. MSTAT-C statistical software package was used for the comparison of the means via the Student–Newman–Keuls test at $p \leq 0.05$.

Results and Discussion

The early planting date (18 March) at the 30 × 2 cm spacing gave significantly higher grain yield than other combinations of planting date and spacing (Figure 1). This shows an increase in the grain yield at the reduced intra-plant spacing of 30×2 cm on this planting. Also, considering the average of the

three plant spacings, the early planting date showed higher biomass than the other dates (April 2 and April 18); however, the difference with the planting date of April 2 was not significant (Table 2). The percentage increase on March 18 and April 2 were 66% and 51% for biomass and 67% and 46% for grain yield, respectively, as compared with the April 18 planting date. The late planting date had a more pronounced negative impact on both grain yield and biomass. Both 20×3 cm and 30×2 cm spacing with higher plant density showed higher biomass and grain yield than the 20×5 cm spacing (Table 2). Early planting could extend the growth period and allow the plant to accumulate more heat units than the late planting on April 18. In consequence, early planting allowed the plant to better utilize soil nutrients and moisture, and accordingly accumulated more carbohydrates in its tissue, which reflected in biomass and economic grain yield (Calleja-Cabrera *et al.* 2020; Prusiński and Nowicki 2020; Lee *et al.* 2022; Lin *et al.* 2024).

The data showed that changing the plant spacing influenced both grain yield and biomass, which were increased by increasing plant density. The increase in grain yield and biomass could be due to the higher number of plants per unit area and lower plant-to-plant competition (Ouji *et al.* 2021; Rabbani *et al.* 2022; Rabbani *et al.* 2023). Narrow spacing is chosen most often to increase photosynthetic capacity by increasing the interception of solar radiation, and consequently results in improved yield (Liu *et al.* 2016; Zhang *et al.* 2022). Our results suggest that *L. iberica* can be grown successfully at 20×3 cm and 30×2 cm spacing.

Essential oils and their components

Planting date affected the essential oil percentage and yield of *L. Iberica*, which were higher on March 18 than on other dates. Spacing also affected the essential oil yield, and the highest amount was observed in the 20×3 and 30×2 cm plant spacing (Table 2). Thus, the planting of *L. iberica* is preferred in March because it provides a longer growing season to take advantage of the environmental sources. It also helps the plant to avoid the unsuitable environmental conditions of later dates. As is known, synthesis of essential oil is influenced by the stresses such as high temperature during the seed filling period, whereby the crop at the late planting date had a lower essential oil percentage than other dates (Table 2). However, some studies (Ebrahimghochi *et al.* 2018; Hüsni Can Başer and Buchbauer 2021) have shown that the delayed planting dates, followed by the longer days and high temperature, increased essential oil percentage.

In this study, 23 essential oil components were identified. The percentages of essential oil components are shown in Table 3. Caryophyllene oxide, spathulenol, carvacrol, thymol, phytol, and trans-caryophyllene were the major components of essential oil. Considering the average of three

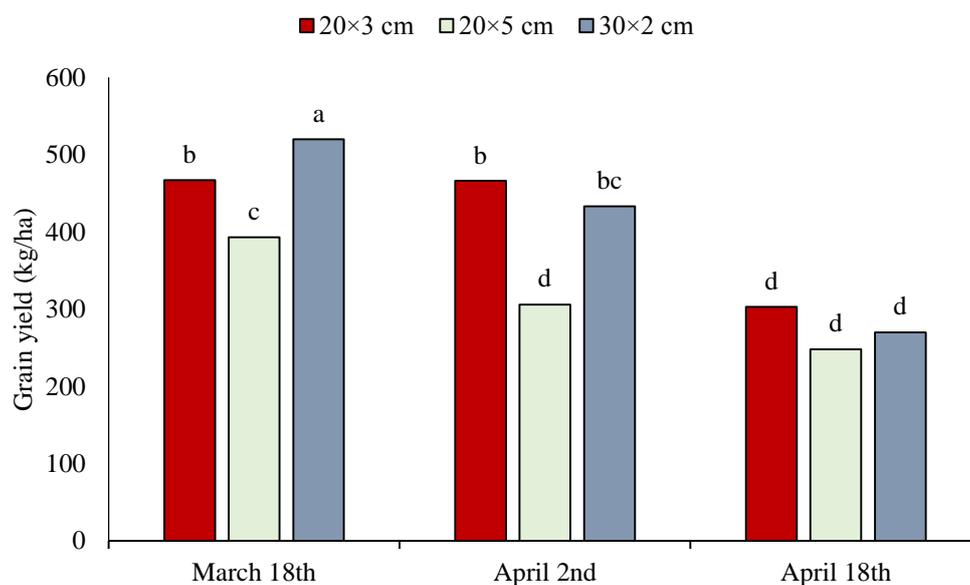


Figure 1. Grain yield of *Lallelantia iberica* affected by planting date and spacing interaction; Means followed by different letters are significantly different at $p \leq 0.05$, according to the Student–Newman–Keuls test.

Table 2. Effect of different planting dates and spacing on some traits of *Lallelantia iberica*.

Treatment	Biomass (kg/ha)	Essential oil (%)	Essential oil yield (kg/ha)	Mucilage (%)	Mucilage yield (kg/ha)	N (%)	P (%)	K (%)
Planting date								
March 18	3796 ^a	0.34 ^a	13.17 ^a	15.30 ^a	70 ^a	1.70 ^a	0.19 ^a	4.25 ^a
April 2	3470 ^a	0.32 ^{ab}	11.14 ^b	13.58 ^b	55 ^b	1.55 ^a	0.16 ^b	3.64 ^b
April 18	2286 ^b	0.30 ^b	7.06 ^c	12.06 ^c	33 ^c	1.53 ^a	0.16 ^b	3.54 ^b
Plant spacing (cm)								
20 × 3	3574 ^a	0.32 ^a	11.53 ^a	13.76 ^a	57 ^a	1.53 ^b	0.16 ^b	3.70 ^b
20 × 5	2428 ^b	0.32 ^a	7.88 ^b	13.94 ^a	44 ^b	1.82 ^a	0.20 ^a	4.10 ^a
30 × 2	3550 ^a	0.31 ^a	11.98 ^a	13.51 ^a	56 ^a	1.45 ^b	0.15 ^b	3.63 ^b

Means in each column for each factor followed by the different letters are significantly different at $p \leq 0.05$, according to the Student–Newman–Keuls test.

plant spacings, sowing in April 18 with 7.91% for thymol, 29.52% for caryophyllene oxide, 5.99% for α -bisabolol, and 7.18% for phytol showed the highest amount in comparison to other planting dates. The highest values for trans-caryophyllene (5.13%) and spathulenol (12.41%) were obtained at the April 2 planting date. The highest content of linalool and carvacrol, with 3.65% and 6.27%, respectively, was gained on March 18. Moreover, the highest percentage of linalool, carvacrol, and trans-caryophyllene was obtained at the 20×5 cm spacing for all three planting dates. The caryophyllene oxide, which had the highest percentage among essential oils, showed the highest

percentage at the spacing of 20×3 cm and 30×2 cm at all sowing dates. Generally, the highest values of the most important compounds in essential oil were obtained from the *L. iberica* plants, which were planted on April 18 with 30×2 cm and 20×3 cm spacing.

Table 3. Effect of planting date and spacing on essential oil composition (%) of *Lallemantia iberica*.

Compounds	RI	RT	March 18			April 2			April 18		
			20×3	20×5	30×2	20×3	20×5	30×2	20×3	20×5	30×2
Limonene	1037	7.24	-	-	-	-	-	-	-	2.01	-
Linalool	1099	8.80	3.50	3.90	3.54	2.59	3.26	2.78	2.61	3.44	2.43
Geraniol	1253	12.36	-	3.86	-	-	3.18	-	1.68	2.08	1.73
Trans-anethole	1287	13.12	-	-	-	-	-	-	1.67	-	1.52
Thymol	1291	13.21	4.22	2.28	4.29	3.68	2.39	3.79	7.39	8.85	7.50
Carvacrol	1299	13.41	-	6.27	-	-	2.53	-	1.75	2.72	1.67
β. bourbonene	1389	15.40	2.68	-	2.72	-	-	-	-	-	-
Trans-caryophyllene	1425	16.15	3.69	6.68	3.77	4.68	6.00	4.71	3.61	4.14	3.45
Germacrene-d	1487	17.44	2.70	2.72	2.77	4.04	-	4.13	1.32	-	1.36
δ-cadinene	1527	18.24	3.10	3.57	2.28	-	3.16	-	-	2.13	-
β-calacorene	1559	18.88	3.01	3.42	3.10	1.43	3.63	1.25	4.97	3.37	5.58
Germacrene-D-4-ol	1574	19.17	2.40	2.25	2.42	2.65	2.27	2.79	2.21	-	2.01
Spathulenol	1586	19.41	8.79	7.61	8.87	14.58	7.57	15.08	12.74	10.00	12.41
Caryophyllene oxide	1592	19.53	28.45	24.4	28.24	28.31	25.55	29.28	31.01	27.12	30.42
1,10-di-epi-cubenol	1616	19.99	3.28	4.48	3.34	1.57	4.22	1.62	1.67	3.25	1.15
α-Cadinol	1658	20.80	-	-	-	9.34	-	9.15	-	-	-
Epi-α-bisabolol	1687	21.35	3.50	2.81	3.61	5.67	3.57	5.50	5.97	5.40	6.59
Pentadecanoic acid	1872	25.06	3.11	-	3.02	-	-	-	-	3.00	-
Hexadecanoic acid	1967	26.55	-	3.80	-	1.75	3.99	1.86	3.49	-	3.98
Nonacosane	2068	28.17	-	4.56	-	1.59	4.51	1.61	1.56	3.11	1.67
Phytol	2110	28.46	4.64	2.06	4.49	5.63	3.32	5.59	7.45	5.40	8.69
Methyl octadecanoate	2134	28.71	3.62	-	3.54	-	-	-	-	-	-
Heptacosane	2234	29.74	3.21	-	3.33	-	-	-	-	-	-

RI: Retention indices, RT: Retention time.

Oil and fatty acids

The highest and lowest grain oil percentage and yield were obtained on March 18 and April 18, respectively (Figure 2). In the earliest planting date, there was no significant difference among different spacings for the grain oil percent. But, in the later planting dates, the spacing of 20×3 cm showed the highest oil content (27.09% and 24.91% for April 2 and April 18, respectively) in *L. iberica* grains (Figure 2-I). The highest oil yield (165 kg/ha) was obtained from the 30×2 cm spacing

in the earliest planting date, followed by the April 2 planting date. Delaying the planting date decreased oil yield. In addition, on the last planting date, the spacing was ineffective (Figure 2-II).

In all treatments, eight fatty acids were detected. Table 4 shows only the dominant fatty acids, including linolenic acid and linoleic acid as unsaturated fatty acids with multiple bonds, oleic acid as unsaturated fatty acids with a double bond, and palmitic acid and stearic acid as saturated fatty acids. The highest percentage of unsaturated fatty acids (91.41%) was observed on March 18, which showed a slightly decreasing trend in subsequent planting dates. The highest percentage of linolenic acid was obtained on March 18, followed by April 18 at the density of 20×3 cm. Due to the great extent of linolenic acid in *L. iberica* grain oil, this plant's oil is very popular. Saturated fatty acids, including stearic acid and palmitic acid, generally showed the lowest percentage in the March 18 sowing date. On the other hand, the 30×2 cm and 20×5 spacing for April 18 allocated the lowest percentage of total saturated fatty acids.

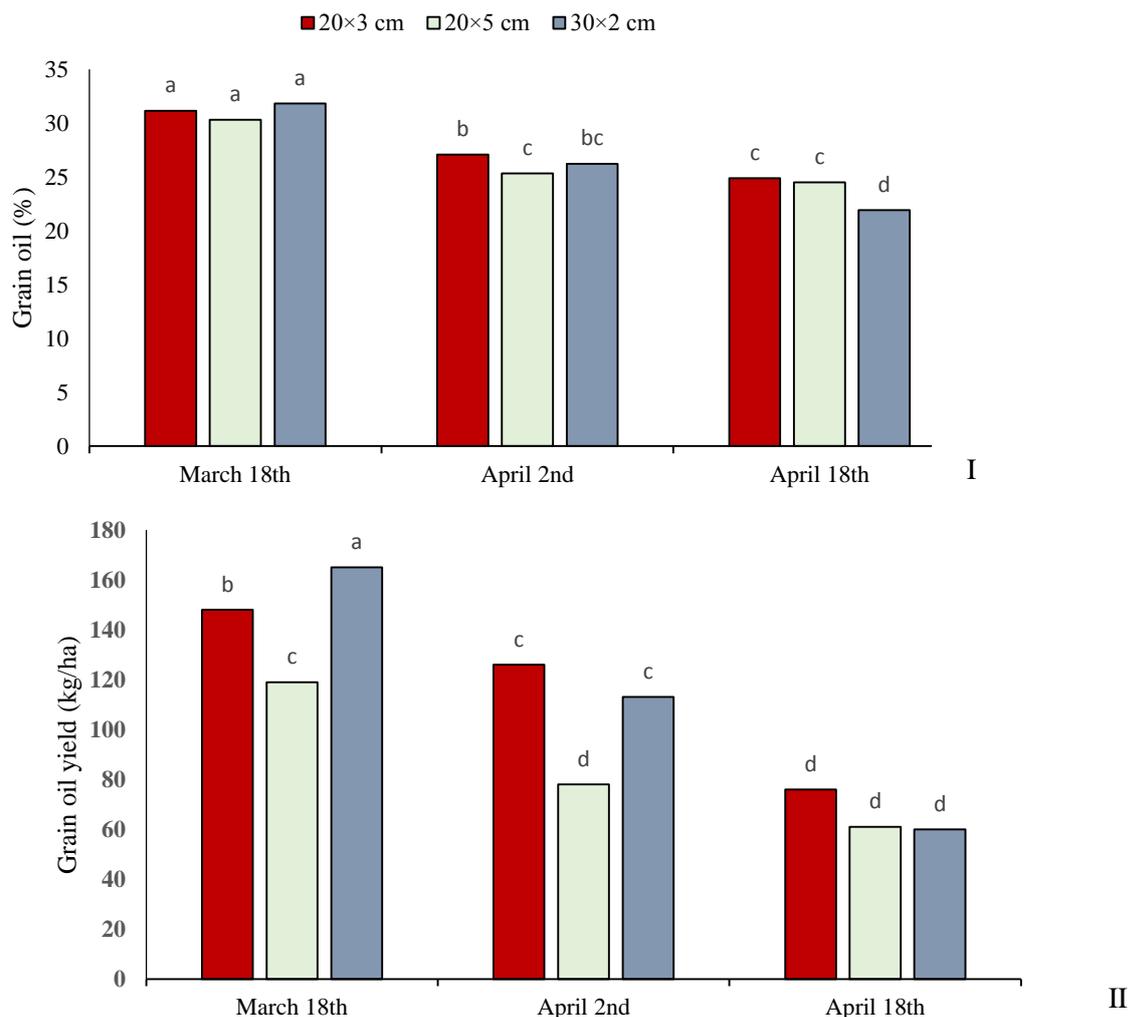


Figure 2. Grain oil (I) and oil yield (II) of *Lallelantia* affected by planting date and spacing interaction; Means followed by different letters are significantly different at $p \leq 0.05$, according to the Student–Newman–Keuls test.

Table 4. The Percentage of fatty acids affected by planting date and spacing.

Planting date	Spacing	Linolenic acid	Linoleic acid	Oleic acid	Total unsaturated fatty acids (%)	Stearic acid	Palmitic acid	Total saturated fatty acids
March18	20×3	65.09	13.05	13/27	91.41	1.21	6.75	7.96
	20×5	63.06	12.78	13.20	89.04	1.65	6.50	8.15
	30×2	64.32	12.68	13.22	90.27	1.22	6.70	7.92
April 2	20×3	63.39	13.09	12.93	89.41	1.28	6.99	8.27
	20×5	63.06	12.97	13.26	89.29	1.77	7.05	8.82
	30×2	63.26	12.55	13.27	89.08	1.81	7.17	8.98
April 18	20×3	65.01	12.01	12.49	89.51	1.39	6.96	8.35
	20×5	58.98	12.47	12.80	84.25	1.49	6.95	8.44
	30×2	60.21	12.28	11.91	84.40	1.23	6.92	8.15

Mucilage

Another quality characteristic that was studied was mucilage percentage and yield. Sowing date had a significant effect on these traits. The highest percentage of mucilage (15.3 %) was obtained at the earliest sowing date (March 18). The highest yield of mucilage (70 kg/ha) was also obtained from the first planting date. By delaying planting, the mucilage percentage exhibited a downward trend. Different spacings did not affect the percentage of mucilage. However, the 20×3 cm spacing showed the highest amount (with 57 kg/ha) of mucilage yield (Table 2). These results agree with the findings of Badawy *et al.* (2013) and Ebrahimi *et al.* (2010) who reported that carbohydrate content increased with the early planting date.

Macro-nutrients that affect NPK absorption

The planting dates affected the P and K content. The highest P (0.19%) and K (4.25%) were obtained on March 18, while they did not affect the nitrogen content (Table 2). However, according to Neenu *et al.* (2017), the delay in sowing might result in the seeds facing a drier soil environment in the later growing times, leading to low nitrogen uptake. There is a positive relationship between K uptake and water acquisition in plants. Hence, the reduction in soil moisture affects the uptake of K and the biomass production (Wang *et al.* 2013). This might be the reason for the low K uptake in late planting dates when there is low rainfall during the crop growing period. Moreover, the more distributed rainfall might supply more soil moisture during the early development of the crop in the earlier sowing

date. In adequate moisture conditions in the soil, the availability of P can also be enhanced (Neenu *et al.* 2017).

Planting spacing affected the percentage of N, P, and K nutrients. Accordingly, the spacing of 20×5 cm with 1.82%, 0.2%, and 4.1% for N, P, and K, respectively, showed the highest amount of these nutrients.

Conclusion

Planting date and spacing showed direct impacts on the quality and quantity of *L. iberica*. In conclusion, sowing on March 18 (early planting) produced higher grain yield and biomass on average of three plant spacing in the dragon's head species. Mutually, late sowing reduced the nutrient uptake, biomass, and some quality traits. Also, the spacing of 30 × 2 cm with lower density showed better results. The quality of grain oil was slightly improved by the earlier planting date (March 18) due to a reduction in the proportion of total saturated fatty acids. However, at the April 18 planting date, the 30 × 2 cm spacing caused an increase in the most important compounds of essential oil, including caryophyllene oxide and spathulenol. This study opens a path for further specific studies on proper agronomical conditions to reach the best performance of this economic plant.

Conflict of Interest

The authors declare that they have no known competing interests with any individuals or organizations concerning the subject of this article.

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